PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Alagu P. THIRUVENGADAM, et al.

Docket No: A8709

Appln. No.: 10/823,647

Group Art Unit: 1651

Confirmation No.: 4915

Examiner: Taeyoon KIM

Filed: April 14, 2004

For: METHODS FOR DIAGNOSING A BIPOLAR DISORDER AND UNIPOLAR DISORDER

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Alagu P. Thiruvengadam, hereby declare and state:

THAT I am a citizen of the United States of America;

THAT I have received a Ph.D. degree in 1962 from Indian Institute of Science;

THAT I am a named inventor of the application;

THAT I am familiar with the disclosure and claims of the above-identified patent application;

THAT I am also familiar with the Office Action dated May 31, 2007, in the above-identified application wherein the Examiner rejects claims 27-32 and 45 under 35 U.S.C. § 103, as being unpatentable over El-Mallakh in light of Garrahan or Antia; and

THAT in order to demonstrate the unexpected effects of the claimed invention, the following experimentation was carried out by me or under my supervision:

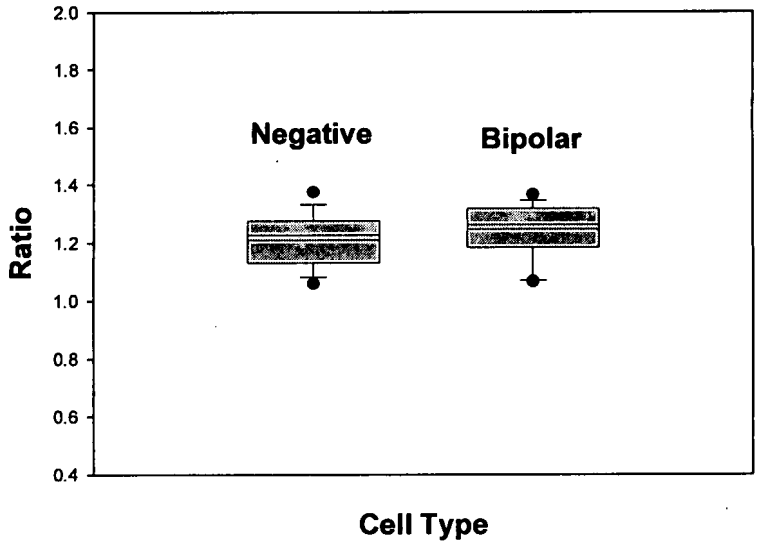
I. Comparison of membrane potential using gramacidin in K^+-containing buffer

Four samples of whole blood cells were incubated with dye for 15 minutes. Two of the samples were drawn from a person unaffected with bipolar disease (i.e., the negative cells). Two of the samples were drawn from a person affected with bipolar disease (i.e., the bipolar cells). For each of the samples, thirty micro-liters (30 µl) of whole blood cells were added to buffers containing potassium buffer (5mM KCl, 4 mM $NaHCO_3$, 5mM HEPES, 134 mM NaCl, 2.3 mM $CaCl_2$, and 5 mM glucose). The dye (3,3'-dihexyloxacarbocyanine) was added thereafter. Ten micro-liters (10 µl) of 2mM gramicidin per ml of buffer were added to one sample of the negative cells and to one sample of the bipolar cells. The other samples were untreated with gramacidin.

The membrane potential was measured in each of the four samples. Each well of a 96 well plate was filled with two hundred micro liters (200 µl) of the samples. The 96 well plate was tested in a plate reader (Perkins-Elmer Victor II) for membrane potentials.

The ratios were calculated for each of negative cells and bipolar cells. The membrane potentials between the gramicidin treated sample and the untreated sample for negative cells were used to calculate the ratio for the negative cells. The membrane potentials between the gramicidin treated sample and the untreated sample for bipolar cells were used to calculate the ratio for the bipolar cells. The ratios obtained with negative cells and bipolar cells were compared and are shown in the figure below.

Negative vs Bipolar
Gramicidin Protocol
Not Significant



t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.818)

Group Name	N	Missing	Mean	Std Dev	SEM
Control	24	0	1.210	0.0928	0.0189
Bipolar	24	0	1.246	0.0947	0.0193

Difference -0.0357

t = -1.318 with 46 degrees of freedom. (P = 0.194)

95 percent confidence interval for difference of means: -0.0902 to 0.0188

As shown above, no significant difference between control and bipolar samples was observed. In this regard, the experiment shows that the use of gramicidin and K^+-containing buffer for each of the samples does not provide for a significant difference in membrane potential between bipolar cells and negative cells.

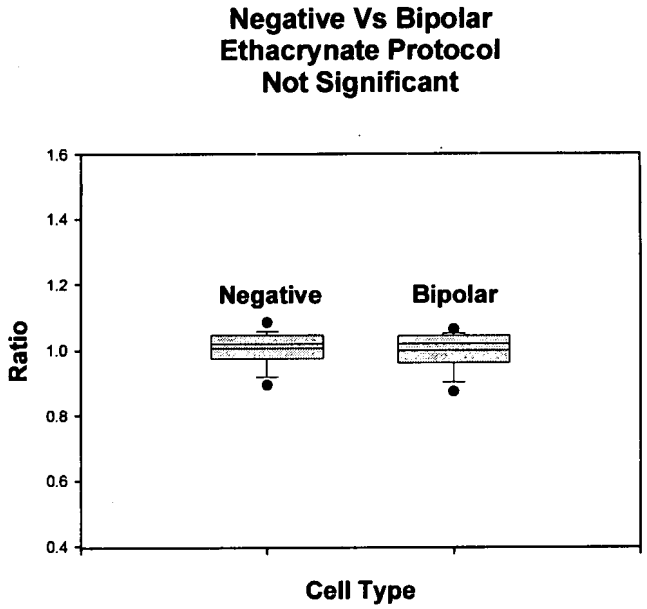
II. Comparison of membrane potential using ethacrynate in K^+-containing buffer

Four samples of blood cells were incubated with dye for 15 minutes. Two of the samples were drawn from a person unaffected with bipolar disease (i.e., the negative cells). Two of the samples were drawn from a person affected with bipolar disease (i.e., the bipolar cells). For each of the samples, thirty micro-liters (30 µl) of whole blood cells were added to buffers containing potassium buffer (5mM KCl, 4 mM $NaHCO_3$, 5mM HEPES, 134 mM NaCl, 2.3 mM $CaCl_2$, and 5 mM glucose). The dye (3,3'-dihexyloxacarbocyanine) was added thereafter. Ethacrynate was added to one sample of the negative cells and to one sample of the bipolar cells to amount to a concentration of one micro-mole (1mM) in each of the two samples. The other samples were untreated with ethacrynate.

The membrane potential was measured in each of the four samples. Each well of a 96 well plate was filled with two hundred micro liters (200 µl) of the samples. The 96 well plate was tested in a plate reader (Perkins-Elmer Victor II) for membrane potentials.

The ratios were calculated for each of negative cells and bipolar cells. The membrane potentials between the gramicidin treated sample and the untreated sample for negative cells were used to calculate the ratio for the negative cells. The membrane potentials between the gramicidin treated sample and the untreated sample for bipolar cells were used to calculate the

ratio for the bipolar cells. The ratios obtained with negative cells and bipolar cells were compared and are shown in the figure below.



Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Control	24	0	1.019	0.973	1.044
Bipolar	24	0	1.018	0.964	1.041

T = 615.000 n(small)= 24 n(big)= 24 (P = 0.585)

As shown above, no significant difference between control and bipolar samples was observed. In this regard, the experiment shows that the use of ethacrynate and K^+-containing buffer for each of the samples does not provide for a significant difference in membrane potential between bipolar cells and negative cells.

It is evident from the foregoing results that the claimed method is unexpectedly superior over the methods disclosed in the cited art. In contrast to the above experiments, the claimed method provides for a significant difference in membrane potential between bipolar cells and negative cells.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7/31/07

Alagu P. Thiruvengadam